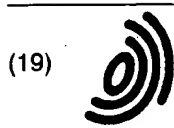


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(54) **DHA-PHARMACEUTICAL AGENT CONJUGATES**

DHA-PHARMAZEUTISCHES PRODUKT KONJUGATE

CONJUGUES D'ACIDE CIS-DOCOSAHEXANOIQUE ET D'AGENTS PHARMACEUTIQUES

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(56) References cited:
WO-A-89/07938 **WO-A-92/20362**
WO-A-94/12530 **WO-A-96/04001**
WO-A-96/21658 **WO-A-97/44026**
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Description

Background of the Invention

[0001] Improving drug selectivity for target tissue is an established goal in the medical arts. In general, it is desirable to deliver a drug selectively to its target, so that dosage and, consequently, side effects can be reduced. This is particularly the case for toxic agents such as anti-cancer agents because achieving therapeutic doses effective for treating the cancer is often limited by the toxic side effects of the anti-cancer agent on normal, healthy tissue. The problems relating to lack of drug selectivity can be exemplified by Taxol®.

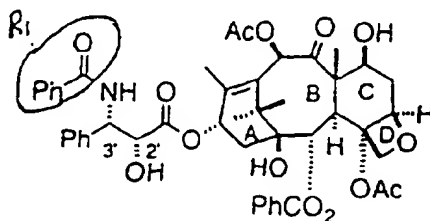
[0002] Taxol® (paclitaxel) was first isolated in 1971 from the bark of *Taxus brevifolia* and was approved in 1992 by the US Food and Drug Administration for treatment of metastatic ovarian cancer and later for breast cancer. Its mechanism of action is believed to involve promoting formation and hyperstabilization of microtubules, thereby preventing the disassembly of microtubules necessary for completion of cell division. It also has been reported that Taxol induces expression of cytokines, affects the activity of kinases and blocks processes essential for metastasis, in as yet uncharacterized mechanisms of action.

[0003] Taxol has attracted unusually strong scientific attention, not only because of its unique antiproliferative mechanism of action, but also because it is active against nearly all cancers against which it has been tested and because it has been discovered to be an analog of numerous closely related compounds occurring naturally. These compounds, taxanes, are now recognized as a new class of anticancer compounds.

[0004] Taxol's strength against cancers of diverse tissue origin also represents a significant drawback. An ideal anticancer agent has tissue specificity, thereby reducing side-effects on normal (dividing) cells. Taxol analogs with tissue specificity therefore are desired. Another drawback of Taxol is its extreme insolubility. Taxol can be administered effectively in a solvent including cremophor, which combination can provoke severe hypersensitive immune responses. As a result of these drawbacks, and also as a result of the potential for modifying Taxol at numerous sites as demonstrated by other naturally-occurring taxanes with anticancer activity, a search for more selective taxanes was launched.

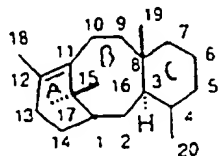
[0005] To date, more than 200 taxanes have been synthesized (or isolated) and tested *in vitro* or *in vivo* for anticancer activity. The results, however, have been so disappointing that the National Cancer Institute (NCI) generally no longer is interested in testing Taxol analogs. In general with Taxol analogs, the solubility problems remain, and/or potency is sharply reduced, and/or selectivity is not improved, and/or the ratio of the median toxic dose to the median effective dose ("therapeutic index") is unacceptably reduced.

[0006] Taxol has the following formula:



[0007] Taxanes have the basic three ring structure (A, B and C), substituted or unsubstituted.

[0008] Taxol's carbons are numbered conventionally as follows:



[0009] Based upon the taxanes tested to date, as many questions have been raised as have been answered, and general rules have not been fashioned easily in predicting selectivity, activity and solubility. Firstly, no rules have emerged regarding selectivity. Those taxanes that are strongly active appear to have activity as broad as Taxol's activity, and no headway appears to have been made in terms of developing a more selective Taxol analog.

[0010] Some information about activity has emerged. Numerous substitutions have been made at C7, C9, C10, C19,

R₁ and combinations thereof while retaining significant, but usually reduced, activity. Substitutions at C2, C4 and 2'OH, however, are generally not tolerated. These conclusions are only generalities, for example, because some substitutions at C9-C10 (cyclic derivatives) are not tolerated and some substitutions at C2 (meta substitutions on the phenyl) are tolerated. Likewise, the C13 side chain and, in particular, the 2'OH are required, although the minimum structural requirements of the side chain have not been determined for therapeutic efficacy.

[0011] Attempts to improve Taxol's solubility have not resulted in successful clinical products. One approach has been to manufacture prodrugs of Taxol, which prodrugs undergo *in vivo* transformation into Taxol and some other product. Attempts were made to esterify the C7 hydroxy and 2' hydroxy groups, with the hope that the bond would be stable in solution (to permit preferred administration modes -i.v. over at least 24 hours) but would cleave readily *in vivo*.

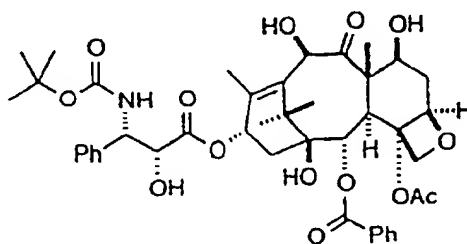
The groups tested were all hydrophilic and included amines, short carboxylic acids (using e.g. succinic anhydride and glutaric anhydride), sulfonic acids, amino acids and phosphates. Generally, activity was reduced although some success was obtained with certain derivatives. Again, no particular pattern emerged permitting one to predict reliably which groups could be substituted on Taxol to yield a therapeutically useful product, although it was suggested that the 2' OH derivatives may cleave more easily than the C7 OH derivatives.

[0012] Several other factors add to the problem of predicting which Taxol analogs will be effective. Multiple mechanisms of action have been proposed in the literature, and a change in one position may have no effect on activity on one such mechanism but may eliminate activity on another mechanism. In addition, changes that favorably influence activity may unfavorably influence bioavailability. For example, Taxol affects microtubule formation inside a cell, but a change in structure that increases intracellular activity may adversely affect the ability of Taxol to gain entry into a cell. Taxol also is known to bind to proteins, and the effect on activity that results from a change in Taxol's binding to protein (in terms of conformation, cellular absorption and solubility) is unknown.

[0013] It has been reported that Taxol does not get into the brain, apparently excluded by the blood brain barrier. It is not known why this is so, as Taxol is lipophilic, gets into cells and might be expected to cross the blood brain barrier.

[0014] Among the most promising of the two hundred analogs tested is Taxotere (docetaxel), because of its slightly increased activity and solubility. Oddly, however, Taxotere differs from Taxol at sites which typically do not have a strong influence on activity, and one would not predict the improvements in Taxotere from these differences, even in hindsight.

[0015] Taxotere has the following formula:



[0016] DHA (docosahexaenoic acid) is a 22 carbon naturally-occurring, unbranched fatty acid that previously has been attached to drugs to help deliver them across the blood brain barrier. DHA is attached via the acid group to hydrophilic drugs and renders these drugs more hydrophobic (lipophilic). DHA is an important constituent of the brain and recently has been approved in Europe as an additive to infant formula. It is present in the milk of lactating women. The mechanism of action by which DHA helps drugs conjugated to it cross the blood brain barrier is unknown.

Summary of the Invention

[0017] The present invention involves the unexpected finding that conjugates of pharmaceutical agents and a highly lipophilic group, a C22 unbranched carbon chain, have a different selectivity relative to the unconjugated pharmaceutical agents. The conjugates, in general, render the activity of these compounds selective for colon tissue, breast tissue and central nervous system tissue ("targeted tissues"). The conjugates, also unexpectedly, restrict the activity of these compounds to cell types within these tissue categories relative to that of the unconjugated pharmaceutical agents. The conjugates, further unexpectedly, reduce sharply the activity of these compounds relative to that of the unconjugated pharmaceutical agents in most cell lines of tissue types other than colon, breast, and central nervous system, thereby reducing potential side effects of the conjugates versus those of the unconjugated pharmaceutical agents. The therapeutic index of the conjugates may be improved, versus that of the unconjugated pharmaceutical agents.

[0018] According to one aspect of the invention, a composition is provided for targeting a taxane to a noncentral nervous system tissue to treat a noncentral nervous system condition. A covalent conjugate of *cis*-docosahexaenoic

acid and a pharmaceutical agent effective for treating said condition is administered to a subject in need of such treatment. Preferably, the conjugate consists only of *cis*-docosahexaenoic acid and the pharmaceutical agent, wherein the *cis*-docosahexaenoic acid is conjugated directly to the pharmaceutical agent, free of linker, for example via the carboxylic acid group of the *cis*-docosahexaenoic acid and a reactive group such as a free amino or hydroxyl group of the pharmaceutical agent. In preferred embodiments, the tissue is breast tissue, gastrointestinal tissue and ovarian tissue and the condition calls for treatment of breast tissue, gastrointestinal tissue or ovarian tissue, respectively.

[0019] The conjugates of the invention can be isolated conjugates. An isolated conjugate is one which is separated from other different docosahexaenoic acid-pharmaceutical agent conjugates.

[0020] The pharmaceutical agent may be any pharmacological compound or diagnostic agent, as desired.

[0021] Included specifically are the taxanes (e.g., Taxol and Taxotere). Conjugates of *cis*-docosahexaenoic acid and taxoids also are embraced by the invention.

[0022] *Cis*-docosahexaenoic acid previously has been conjugated to drugs that are active in the central nervous system. The present invention contemplates the use of *cis*-docosahexaenoic acid in the manufacture of a medicament for treating a noncentral nervous system condition. The invention further contemplates compositions of matter that are covalent conjugates of *cis*-docosahexaenoic acid and noncentral nervous system active pharmaceutical agents. A noncentral nervous system active pharmaceutical agent is one that has no function or use in the central nervous system. Its only therapeutic use is outside of the central nervous system. Examples of such agents include, but are not limited to: Blood glucose regulators, such as tolazamide, tolbutamide, chlorpropamide, acetohexamide, and, glipizide; HMG-coA reductase inhibitors, such as Lovastatin (Mevacor), Simvastatin (Zocor), Pravastatin (Pravachol), and, Fluvastatin (Lescol); Muscosal Protectives, such as Misoprostol (Cytotec); Gastrointestinal motility effectors, such as Cisapride (Propulsid), Metoclopramide (Reglan), and, Hyoscyamine (Levsin); Antidiarrheals, such as Diphenoxylate hydrochloride (Lomotil), Metronidazole (Flagyl), Methylprednisolone (Medrol), and, Sulfasalazine (Azulfidine); and Hormones for treating, inter alia, ovarian conditions, such as Progesterone, Norgestrel, Norethynodrel, Norethindrone, Levonorgestrel, Ethyndiol, Mestranol, Estrone, Equilin, 17 alpha dihydroequilin, equilenin, 17 alpha dihydroequilenin, 17 alpha estradiol, 27 bea estradiol, Leuprolide (Lupron), Testolactone, Clomiphene, urofollitropin, bromocriptine, gonadorelin, danazol, dehydroepiandrosterone, androstenedione, dihydrotestosterone, Relaxin, folliculostatin, Follicle regulatory protein, Gonadocrinins, Oocyte maturation inhibitor, and, Insulin growth factor. Other compounds are detailed below.

[0023] The methods and/or products of the invention are useful for treating a variety of medical conditions including conditions involving abnormal mammalian-cell proliferation. They further are useful in treating diabetes and its complications, excess acid secretion, cardiovascular conditions involving cholesterol (e.g., hyperlipidemia and hypercholesterolemia), diarrhea, ovarian diseases (e.g. endometriosis, ovarian cysts, etc.) and as contraceptive agents. Other conditions treatable according to the invention will be apparent to those skilled in the art based upon the disclosure and lists of compounds provided.

[0024] The methods and/or products of the invention also are useful in treating conditions specific to noncentral nervous system tissue. Such conditions can be specific to breast tissue, gastrointestinal tissue and ovarian tissue. The tissue also may be other noncentral nervous system tissues. Noncentral nervous system tissue includes tissue of the: Blood and Blood Forming system: including platelets, blood vessel wall, and bone marrow; Cardiovascular system: including heart and vascular system; Digestive and excretory system: including alimentary tract, biliary tract, kidney, liver, pancreas and urinary tract; Endocrine system: including adrenal gland, kidney, ovary, pituitary gland, renal gland, salivary gland, sebaceous gland, testis, thymus gland and thyroid gland; Musclar system: including muscles that move the body. Reproductive System: including breast, ovary, penis and uterus; Respiratory system: including bronchus, lung and trachea; Skeletal system: including bones and joints; Tissue, fiber, and integumentary system: including adipose tissue, cartilage, connective tissue, cuticle, dermis, epidermis, epithelium, fascia, hair follicle, ligament, bone marrow, melanin, melanocyte, mucous membrane, skin, soft tissue, synovial capsule and tendon.

Brief Description of the Drawings

[0025]

Figure 1 is a graph plotting concentration of conjugate 1 versus percent growth of leukemia cells.

Figure 2 is a graph plotting concentration of conjugate 1 versus percent growth of non-small cell lung cancer cells.

Figure 3 is a graph plotting concentration of conjugate 1 versus percent growth of colon cancer cells.

Figure 4 is a graph plotting concentration of conjugate 1 versus percent growth of CNS cancer cells.

Figure 5 is a graph plotting concentration of conjugate 1 versus percent growth of melanoma cells.

Figure 6 is a graph plotting concentration of conjugate 1 versus percent growth of ovarian cancer cells.

Figure 7 is a graph plotting concentration of conjugate 1 versus percent growth of renal cancer cells.

Figure 8 is a graph plotting concentration of conjugate 1 versus percent growth of prostate cancer cells.

Figure 9 is a graph plotting concentration of conjugate 1 versus percent growth of breast cancer cells.

Figure 10 is a graph plotting concentration of conjugate 2 versus percent growth of leukemia cells.

Figure 11 is a graph plotting concentration of conjugate 2 versus percent growth of non-small cell lung cancer cells.

Figure 12 is a graph plotting concentration of conjugate 2 versus percent growth of colon cancer cells.

Figure 13 is a graph plotting concentration of conjugate 2 versus percent growth of CNS cancer cells.

Figure 14 is a graph plotting concentration of conjugate 2 versus percent growth of melanoma cells.

Figure 15 is a graph plotting concentration of conjugate 2 versus percent growth of ovarian cancer cells.

Figure 16 is a graph plotting concentration of conjugate 2 versus percent growth of renal cancer cells.

Figure 17 is a graph plotting concentration of conjugate 2 versus percent growth of prostate cancer cells.

Figure 18 is a graph plotting concentration of conjugate 2 versus percent growth of breast cancer cells.

Figure 19 is a graph plotting concentration of Taxol versus percent growth of leukemia cells.

Figure 20 is a graph plotting concentration of Taxol versus percent growth of non-small cell lung cancer cells.

Figure 21 is a graph plotting concentration of Taxol versus percent growth of colon cancer cells.

Figure 22 is a graph plotting concentration of Taxol versus percent growth of CNS cancer cells.

Figure 23 is a graph plotting concentration of Taxol versus percent growth of melanoma cells.

Figure 24 is a graph plotting concentration of Taxol versus percent growth of ovarian cancer cells.

Figure 25 is a graph plotting concentration of Taxol versus percent growth of renal cancer cells.

Figure 26 is a graph plotting concentration of Taxol versus percent growth of prostate cancer cells.

Figure 27 is a graph plotting concentration of Taxol versus percent growth of breast cancer cells.

Detailed Description of the Invention

[0026] *Cis*-docosahexaenoic acid (DHA) is a naturally occurring fatty acid. It is an unbranched chain fatty acid with six double bonds, all *cis*. Its structure is as follows:



[0027] DHA can be isolated, for example, from fish oil or can be chemically synthesized. These methods, however, can generate *trans* isomers, which are difficult and expensive to separate and which may present safety problems in humans. The preferred method of production is biological synthesis to produce the all *cis* isomer. The preferred source of DHA is from Martek Biosciences Corporation of Columbia, Maryland. Martek has a patented system for manufacturing DHA using microalgae which synthesize only a single isomer of DHA, the all *cis* isomer. Martek's patents include U.S. Pat. Nos. 5,374,657, 5,492,938, 5,407,957 and 5,397,591.

[0028] DHA also is present in the milk of lactating women, and Martek's licensee has obtained approval in Europe of DHA as a nutritional supplement for infant formula.

[0029] It is known that DHA can be unstable in the presence of oxygen. To stabilize DHA and its conjugates it is important to add anti-oxidants to the material after it is synthesized. One method of stabilization is to make-up the newly synthesized material in the following solution:

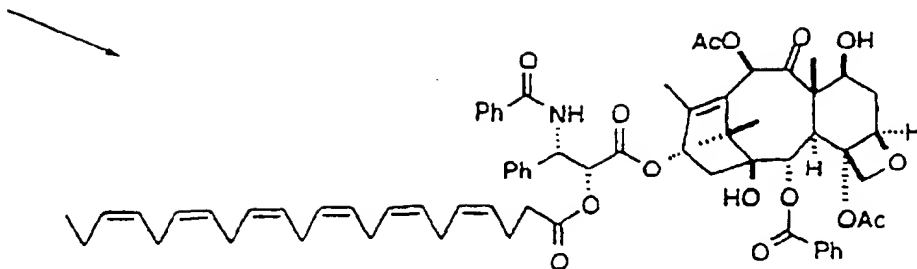
100 g neat DHA-taxol plus 100 g of vehicle (100ml propylene glycol, 70 mg α -tocopherol, 5 mg dialaurylthiodi-propionic acid, 50 mg ascorbic acid) prepared and held under argon in amber, sealed vials and stored at four degrees centigrade. The following anti-oxidants may also be employed: ascorbic acid, ascorbyl palmitate, dialauryl ascorbate, hydroquinone, butylated hydroxyanisole, sodium meta bisulfite, β -carotene and α -tocopherol. A heavy metal chelator such as ethylenediamine tetra-acetic acid (EDTA) may also be used.

[0030] Paclitaxel was first isolated from the bark of *Taxus brevifolia* (Wani et al., J. Am. Chem. Soc., 93, 2325, 1971). Its isolation and synthesis have been reported extensively in the literature. Applicants obtained paclitaxel from a commercial source, Hauser Laboratories, of Boulder, Colorado.

Example 1

[0031]

TAXOL

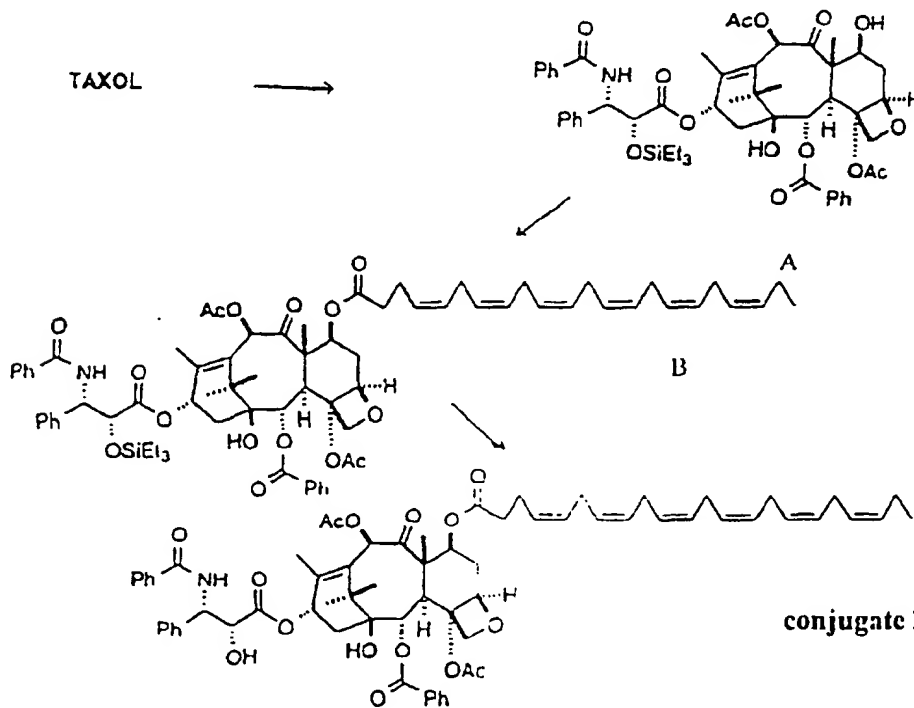
**conjugate 1**

[0032] A solution of Taxol (41 μ mol) in methylene chloride (2.5mL) under argon was mixed with 4-dimethylaminopyridine (41 μ mol), dicyclohexylcarbodiimide (82 μ mol), and DHA (41 μ mol) and the reaction mixture was stirred at ambient temperature for two hours. Following dilution with ether, the reaction mixture was washed with 5% hydrochloric acid, water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue produced 45mg (94%) of crystalline Taxol-DHA conjugate 1.

Example 2

[0033]

TAXOL



[0034] The production of analog 2 involves several steps including a number of protection-acylation-deprotection

steps. A solution of Taxol (59 μ mol) in methylene chloride (2.5mL) was mixed at ambient temperature under argon with imidazole (147 μ mol) and triethylsilyl chloride (147 μ mol). The reaction mixture was stirred for thirty minutes, diluted with additional methylene chloride, washed with water, saturated aqueous sodium chloride, dried, and concentrated. Chromatography of the residue produced 50mg (88%) of intermediate A plus 5mg of the 2', 7-di(triethylsilyl) ether derivative. A solution of intermediate A (52 μ mol) in methylene chloride (3mL) was mixed at ambient temperature under argon with 4-dimethylaminopyridine (52 μ mol), dicyclohexylcarbodiimide (104 μ mol), and DHA (52 μ mol). The reaction mixture was stirred for ten hours, diluted with ether, passed through celite, and concentrated. Chromatography of the residue produced 65.9mg of intermediate B. A solution of intermediate B (51 μ mol) in acetonitrile (2mL) at 0°C under argon was mixed with 49% aqueous HF (0.2mL) and the reaction mixture was stirred for one hour. After dilution with ether, the reaction mixture was washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue produced 44.6mg (75%) of Taxol-DHA conjugate 2.

Example 3

[0035] Conjugates 1 and 2 were sent to the United States National Cancer Institute (NCI) for screening in the NCI's anticancer screening program. The conjugates were provided in ethanol (approximately 40mg analog/2ml ethanol). The conjugates were sealed in vials under argon to avoid exposure of the conjugates to oxygen because the conjugates were believed to be sensitive to oxygen. Instructions were provided to store at 4°C and to open the vials only when ready for immediate experimental use. Instructions also were provided to use the ethanol solutions containing the conjugates directly or to dissolve the analogs further in DMSO (dimethylsulfoxide) at appropriate concentrations, with vortexing if necessary for adequate dispersal.

[0036] The activities of conjugates 1 and 2 were tested against 57 cancer cell lines. The results are presented in Figs. 1-9 for conjugate 1, Figs. 10-18 for conjugate 2 and Figs 19-27 for Taxol. To understand the data, reference is made to the guides provided by the NCI, excerpted as follows:

The Calculated Measurement of Effect: Percentage Growth (PG)

[0037] The measured effect of the compound on a cell line is currently calculated according to one or the other of the following two expressions:

If $(\text{Mean OD}_{\text{test}} - \text{Mean OD}_{\text{zero}}) \geq 0$, then

$$\text{PG} = 100 \times (\text{Mean OD}_{\text{test}} - \text{Mean OD}_{\text{zero}}) / (\text{Mean OD}_{\text{ctrl}} - \text{Mean OD}_{\text{zero}})$$

If $(\text{Mean OD}_{\text{test}} - \text{Mean OD}_{\text{zero}}) < 0$, then

$$\text{PG} = 100 \times (\text{Mean OD}_{\text{ctrl}} - \text{Mean OD}_{\text{zero}}) / \text{Mean OD}_{\text{zero}}$$

Where:

Mean OD_{zero} = The average of optical density measurements of SRB (Sulforhodamine B)-derived color just before exposure of cells to the test compound.

Mean OD_{test} = The average of optical density measurements of SRB-derived color after 48 hours exposure of cells to the test compound.

Mean OD_{ctrl} = The average of optical density measurements of SRB-derived color after 48 hours with no exposure of cells to the test compound.

[0038] Experimental data was collected against each cell line... Each concentration is expressed as the log₁₀ (molar or μ g/ml)... The response parameters G150, TGI (Total Growth Inhibition) and LC50 are interpolated values representing the concentrations at which the PG is + 50, 0, and -50 respectively. Sometimes these response parameters cannot be obtained by interpolation. If, for instance, all of the PGs in a given row exceed +50, then none of the three parameters

can be obtained by interpolation. In such a case, the value given for each response parameter is the highest concentration tested.... This practice is extended similarly to the other possible situations where a response parameter cannot be obtained by interpolation.

5 Dose-Response Curves:

[0039] The dose-response curve page of the data package is created by plotting the PGs against the \log_{10} of the corresponding concentration for every cell line. The cell line curves are grouped by subpanel. Horizontal lines are provided at the PG values of +50, 0, and -50. The concentrations corresponding to points where the curves cross these

10 lines are the GI50, TGI and LC50, respectively.

[0040] Several important distinctions are apparent from the data. Most important, the patterns of anticancer activity for conjugates 1 and 2 differ from that of Taxol. In one sense, conjugates 1 and 2 are effective anticancer agents against a more restricted set of cancer cell lines. For example, conjugates 1 and 2 were not very effective against any of the six leukemia cancer cell lines tested, whereas Taxol was somewhat effective against all four leukemia cell lines against

15 which Taxol was tested. (See Figs. 1, 10 and 19.)

[0041] The relative activity against members within a class of cancers also was altered. For example, at TGI (horizontal line at zero in the graphs), Taxol was more effective against non-small cell lung cancer line H522 than against H460 (by about 3 logs), whereas conjugates 1 and 2 were slightly more effective against H460 than H522. As another example, Taxol was least effective at TGI against CNSU251, whereas conjugate 1 was most effective against CNSU251

20 and conjugates 2 was also very effective against CNSU251 (relative to other CNS cell lines). As a further example, Taxol was equivalent in activity toward MDA-N and MDA-MB-435 breast cancer cell lines at all concentrations tested, whereas conjugates 1 and 2 were more effective against MDA-N than MDA-MB-435 at all concentrations tested.

[0042] To further illustrate the differences in the activity of conjugates 1 and 2 versus that of Taxol, the NCI subjected the data to a statistical analysis designed by the NCI to reflect differences in the pattern of activity of anticancer agents. Conjugate 1 and conjugate 2 were determined to be statistically different in their pattern of activity versus Taxol in this

25 unique measurement by the NCI.

[0043] It also is to be noted that, in general, conjugates 1 and 2 were one thousand to ten thousand times less potent than Taxol for many cell lines tested. This reduction in activity is important, especially since conjugates 1 and 2 maintained strong activity against some cell lines. Conjugates 1 and 2 will be sufficiently active against certain cell lines, but will have, on average, a substantially and disproportionately lower activity against other cell lines, reducing potential side effects. For example, the TGI for Taxol against CNS SF-539 is -6.95, and the TGI for conjugate 1 against this cell line is -5.13 and for conjugate 2 is -5.53. (In other words, the activity of the conjugates was reduced versus that of Taxol by less than 2 logs). The GI50 for Taxol against CNS SF 539 is -7.52, whereas the GI50s for conjugates 1 and 2 are -6.22 and -5.56, respectively (again less than 2 logs difference). In contrast, Taxol has a GI50 for cell line CNSSF 268 of less than -10.0, whereas conjugates 1 and 2 have GI50s for CNSSF 268 of 5.36 and 5.28, respectively. This represents a reduction of activity in the conjugates vs. that of Taxol by at least about 5 logs activity! On average, the GI50 for Taxol across all cell lines tested is at least -9.19. (It is probably much higher since concentrations less than -10 were not tested, and if Taxol was active at -10.0, -10 (instead of the actual lower value) was used in calculating the average of -9.19. There were 27 instances when this occurred.) The average GI50s for conjugates 1 and 2, on the

40 other hand, were 5.49 and 5.22, respectively. Therefore, the average difference in activity for Taxol vs. the conjugates is at least between 3 and 4 logs. Thus, the sharp reduction in the activity of the conjugates against many cell lines vs. a lesser reduction for other cell lines is expected to reduce the potential side effects of the conjugates versus those of Taxol at effective doses.

[0044] Cancers other than CNS, breast and colon cancer can be treated. For example, there was activity against non-small cell lung cancer cells, melanoma cells and ovarian cancer cells. However, the activity was relatively reduced and was extremely specific, limiting the utility of the conjugates for treating generally such cancers. In any event, cancer patients could be evaluated to determine if a conjugate is strongly active against the patient's cancer prior to selecting the conjugate as the anti-cancer agent of choice for that patient.

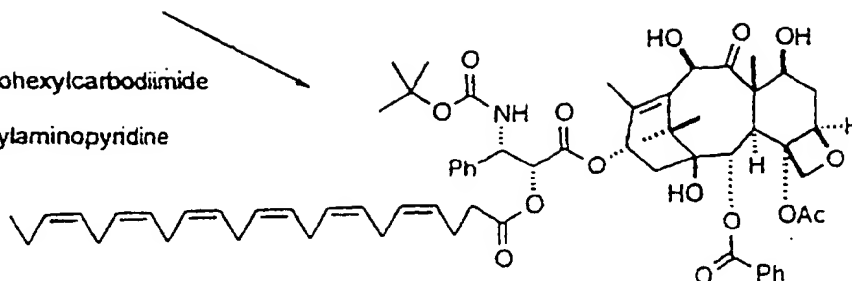
[0045] The foregoing experiments establish that DHA analogs have altered specificity versus that of Taxol for cancer cell lines. Because of this altered specificity, it also is clear that the conjugates themselves are gaining access into the target cells (as opposed to simply releasing Taxol into the environment outside of the cell). Thus, the DHA moiety appears to selectively target certain cell types as opposed to others. The ability of the conjugates to gain entry into the cells was unknown prior to the invention, and the ability of the DHA moiety to selectively target certain cell types was unexpected.

55 [0046] The same is true of DHA-Taxotere covalent conjugates, examples of which are presented below. Taxotere's synthesis has been reported extensively in the literature. One example is Kanazawa, A. et al., *J. Organic Chem.* 1994, Vol. 59. pp. 1238-1240.

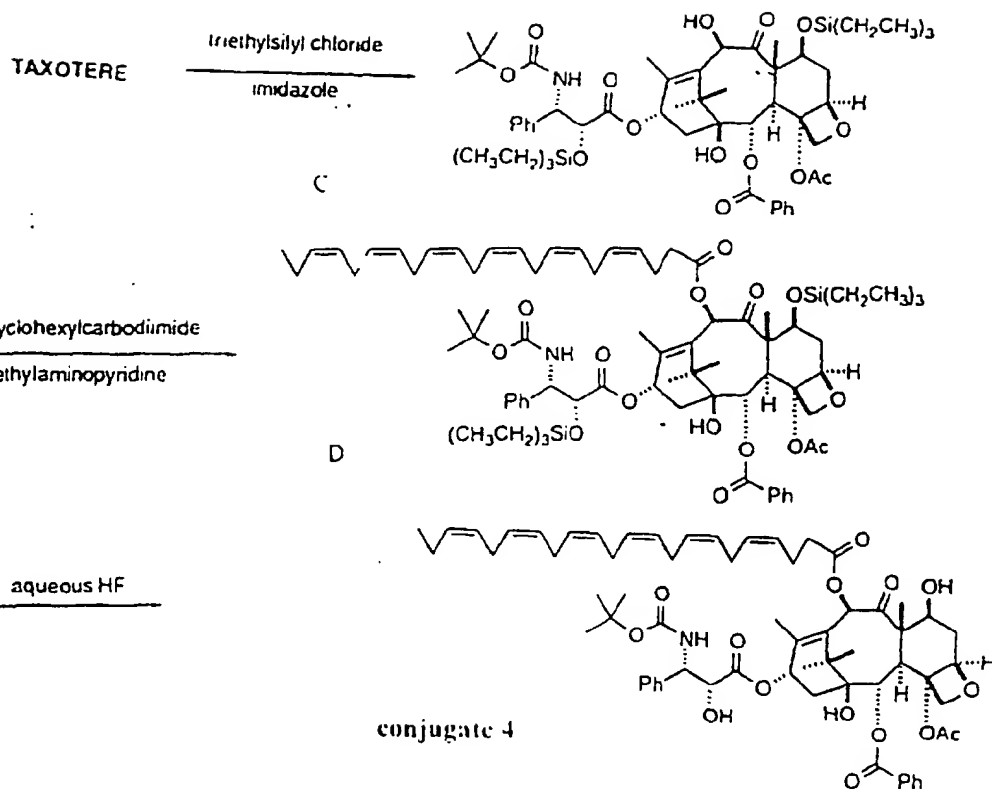
Example 4**[0047]****TAXOTERE**

DHA / dicyclohexylcarbodiimide

4-dimethylaminopyridine

**conjugate 3**

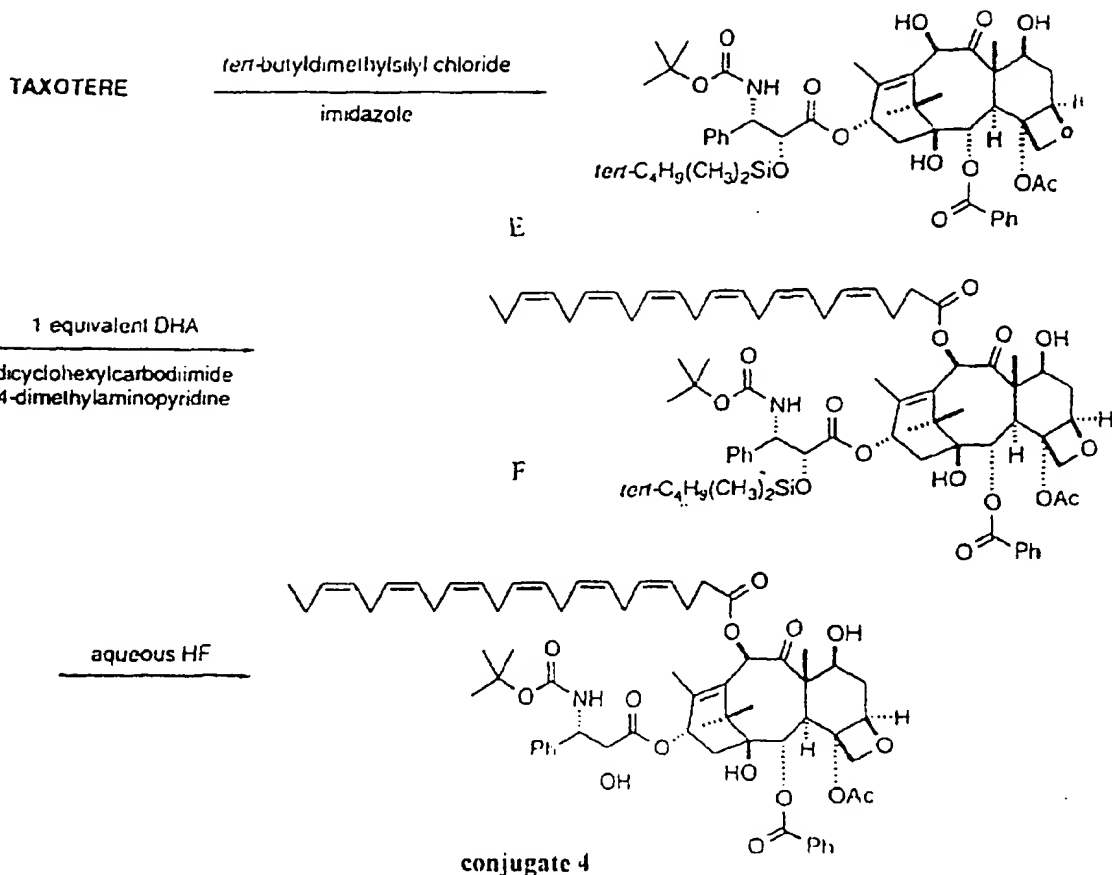
[0048] A solution of Taxotere in methylene chloride under argon is mixed with 4-dimethylaminopyridine, dicyclohexylcarbodiimide, and DHA. The reaction mixture is stirred at ambient temperature. Radial chromatography of the residue is performed to produce Taxotere-DHA conjugate 3.

Example 5**[0049]**

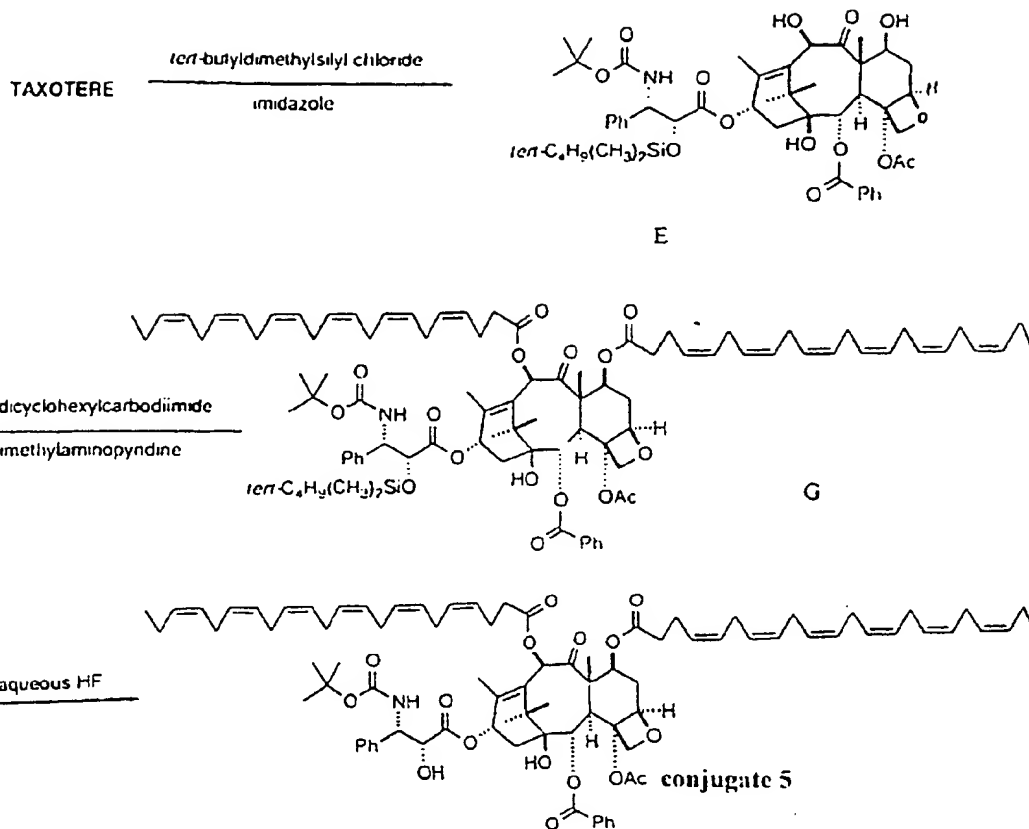
[0050] A solution of Taxotere in dimethylformamide is mixed at ambient temperature under argon with imidazole and triethylsilyl chloride. The reaction mixture is stirred at ambient temperature, diluted with methylene chloride, washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue is performed to produce intermediate C. A solution of intermediate C in methylene chloride is mixed at ambient temperature under argon with 4 dimethylaminopyridine, dicyclohexylcarbodiimide, and DHA. The reaction mixture is stirred at ambient temperature, diluted with ether, passed through celite, and concentrated. Radial chromatography of the residue is performed to produce intermediate D. A solution of intermediate D in acetonitrile at 0°C under argon is mixed with 49% aqueous HF and the reaction mixture is stirred at the same temperature. After dilution with ether, the reaction mixture is washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue is performed to produce Taxotere-DHA conjugate 4.

Example 6

[0051]



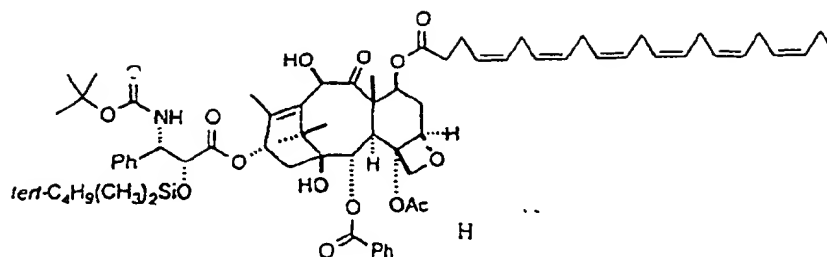
[0052] A solution of Taxotere in dimethylformamide is mixed at ambient temperature under argon with imidazole and *tert*-butyldimethylsilyl chloride. The reaction mixture is stirred at ambient temperature, diluted with methylene chloride, washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue is performed to produce intermediate E. A solution of intermediate E in methylene chloride is mixed at ambient temperature under argon with 4-dimethylaminopyridine, dicyclohexylcarbodiimide, and 1 equivalent of DHA. The reaction mixture is stirred at ambient temperature, diluted with ether, passed through celite, and concentrated. Radial chromatography of the residue is performed to produce intermediate F. (Intermediate H also is obtained and used in Example 8 below.) A solution of intermediate F in acetonitrile at 0°C under argon is mixed with aqueous HF and the reaction mixture is stirred at the same temperature. After dilution with ether, the reaction mixture is washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue is performed to produce Taxotere-DHA conjugate 4.

Example 7**[0053]**

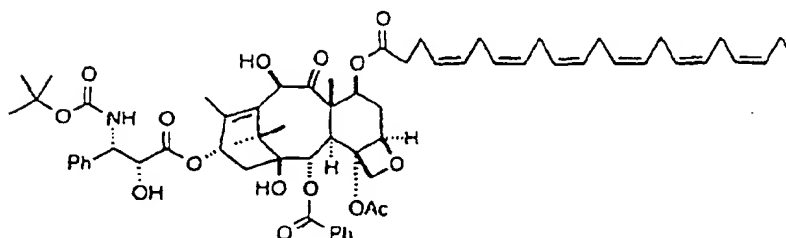
[0054] A solution of Taxotere in dimethylformamide is mixed at ambient temperature under argon with imidazole and *tert*-butyldimethylsilyl chloride. The reaction mixture is stirred at ambient temperature, diluted with methylene chloride, washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue is performed to produce intermediate E. A solution of intermediate E in methylene chloride is mixed at ambient temperature under argon with 4-dimethylaminopyridine, dicyclohexylcarbodiimide, and DHA. The reaction mixture is stirred at ambient temperature, diluted with ether, passed through celite, and concentrated. Radial chromatography of the residue is performed to produce intermediate G. A solution of intermediate G in acetonitrile at 0°C under argon is mixed with aqueous HF and the reaction mixture is stirred at the same temperature. After dilution with ether, the reaction mixture is washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue is performed to produce Taxotere-DHA conjugate 5.

Example 8**[0055]**

1 equivalent DHA

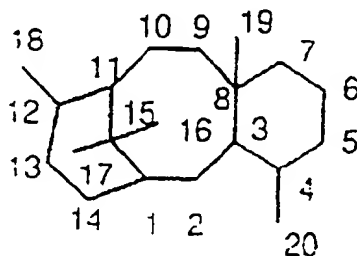
dicyclohexylcarbodiimide
4-dimethylaminopyridine

aqueous HF



[0056] A solution of taxotere in dimethylformamide is mixed at ambient temperature under argon with imidazole and *tert*-butyldimethylsilyl chloride. The reaction mixture is stirred at ambient temperature, diluted with methylene chloride, washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue is performed to produce intermediate E. A solution of intermediate E in methylene chloride is mixed at ambient temperature under argon with 4-dimethylaminopyridine, dicyclohexylcarbodiimide, and 1 equivalent of DHA. The reaction mixture is stirred at ambient temperature, diluted with ether, passed through celite, and concentrated. Radial chromatography of the residue is performed to produce intermediate H (and intermediate F which was used above on Example 6). A solution of intermediate H in acetonitrile at 0°C under argon is mixed with aqueous HF and the reaction mixture is stirred at the same temperature. After dilution with ether, the reaction mixture is washed with water, saturated aqueous sodium chloride, dried, and concentrated. Radial chromatography of the residue is performed to produce Taxotere-DHA conjugate 6.

[0057] As used herein, a taxane is a molecule that possesses the following tricyclic carbon-atom connectivity network, which may incorporate carbon-carbon multiple bonds, and which through the involvement of carbon-atom-noncarbon-atom bonds may include substituents, functional groups, and additional rings.



[0058] A taxoid is a molecule structurally rotated to a taxane in which the above taxane carbon-atom connectivity network is altered, for example, by cleavage of one or more of the carbocyclic rings, by deletion or addition of carbon substituents, by connection of carbon atoms normally not bonded to each other, by disconnection of carbon atoms normally bonded to each other, or by some other reorganization of or adjustment to the taxane carbon-atom connectivity network, but in which one or more structural features characteristic of the taxane carbon-atom connectivity network

are conserved.

[0059] The compounds useful in the invention may be delivered in the form of anti-cancer cocktails. An anti-cancer cocktail is a mixture of any one of the compounds useful with this invention with another anti-cancer agent such as an anti-cancer drug, a cytokine, and/or supplementary potentiating agent(s). The use of cocktails in the treatment of cancer is routine. In this embodiment, a common administration vehicle (e.g., pill, tablet, implant, injectable solution, etc.) would contain both the conjugate useful in this invention and the anti-cancer drug and/or supplementary potentiating agent.

[0060] The compounds of the invention, when used in cocktails, are administered in therapeutically effective amounts. A therapeutically effective amount will be determined by the parameters discussed below, but, in any event, is that amount which establishes a level of the drug(s) in the area of the tumor which is effective in inhibiting the tumor growth.

[0061] When administered, the formulations of the invention are applied in pharmaceutically acceptable amounts and in pharmaceutically acceptable compositions. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic ingredients. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulfonic, tartaric, citric, methane sulfonic, formic, malonic, succinic, naphthalene-2-sulfonic, and benzene sulfonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

[0062] Suitable buffering agents include: acetic acid and a salt (1-2% W/V); citric acid and a salt (1-3% W/V); boric acid and a salt (0.5-2.5% W/V); and phosphoric acid and a salt (0.8-2% W/V).

[0063] Suitable preservatives include benzalkonium chloride (0.003-0.03% W/V); chlorobutanol (0.3-0.9% W/V); parabens (0.01-0.25% WN) and thimerosal (0.004-0.02% WN).

[0064] The active compounds of the present invention may be a pharmaceutical composition having a therapeutically effective amount of a conjugate of the invention optionally included in a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions are capable of being commingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

[0065] Compositions suitable for parenteral administration conveniently comprise a sterile preparation of the conjugates of the invention. This preparation may be formulated according to known methods. Formulations for taxanes can be found in Chapter 9 of Taxol: Science and Applications, CRC Press, Inc., 2000 Corporate Boulevard, N.W., Boca Raton, FL 33431. In general, Taxol has been formulated as a 6 mg/ml cremophor EL (polyoxyethylated castor oil)/ethanol mixture, which is diluted to final volume with normal saline or 5% dextrose. A 15mg/ml solution of taxotere has been formulated in polysorbate 80 (polyoxyethylene sorbitanmonooleate)/ethanol mixture, diluted with 5% dextrose.

[0066] The sterile preparation thus may be a sterile solution or suspension in a non-toxic parenterally-acceptable diluent or solvent. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Carrier formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. can be found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA.

[0067] A subject as used herein means humans, primates, horses, cows, pigs, sheep, goats, dogs, cats and rodents.

[0068] The conjugates of the invention are administered in effective amounts. An effective amount means that amount necessary to delay the onset of, inhibit the progression of, halt altogether the onset or progression of or diagnose the particular condition being treated. In general, an effective amount for treating cancer will be that amount necessary to inhibit mammalian cancer cell proliferation in-situ. When administered to a subject, effective amounts will depend, of course, on the particular condition being treated; the severity of the condition; individual patient parameters including age, physical condition, size and weight; concurrent treatment; frequency of treatment; and the mode of administration. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment.

[0069] Dosage may be adjusted appropriately to achieve desired drug levels, locally or systemically. Generally, daily oral doses of active compounds will be from about 0.01 mg/kg per day to 1000 mg/kg per day. It is expected that IV doses in the range of about 1 to 1000 mg/m² per day will be effective. In the event that the response in a subject is insufficient at such doses, even higher doses (or effective higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Continuous IV dosing over, for example 24 hours or multiple doses per day are contemplated to achieve appropriate systemic levels of compounds.

[0070] A variety of administration routes are available. The particular mode selected will depend of course, upon the particular drug selected, the severity of the disease state being treated and the dosage required for therapeutic efficacy. The methods of this invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, sublingual, topical, nasal, transdermal or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intramuscular, or infusion, intravenous routes are preferred.

[0071] The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the conjugates of the invention into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the compounds into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

[0072] Compositions suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets, or lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquors or non-aqueous liquids such as a syrup, an elixir, or an emulsion.

[0073] Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the active compounds of the invention, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer based systems such as polylactic and polyglycolic acid, polyanhydrides and polycaprolactone; nonpolymer systems that are lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-, di and triglycerides; hydrogel release systems; silastic systems; peptide based systems; wax coatings, compressed tablets using conventional binders and excipients, partially fused implants and the like. In addition, a pump-based hardware delivery system can be used, some of which are adapted for implantation.

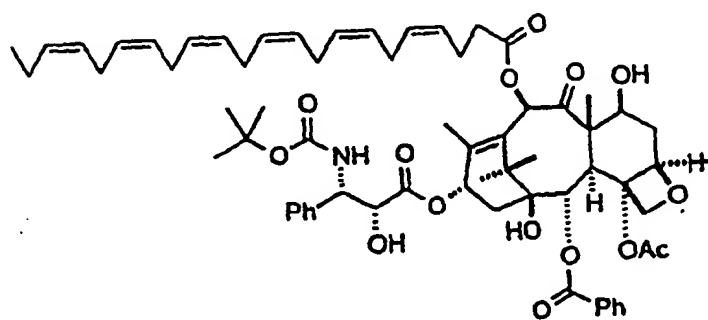
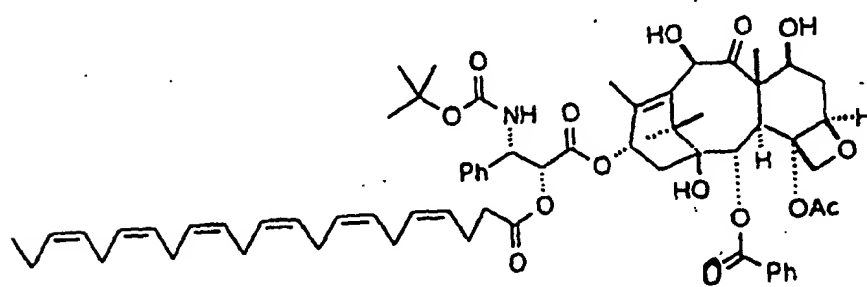
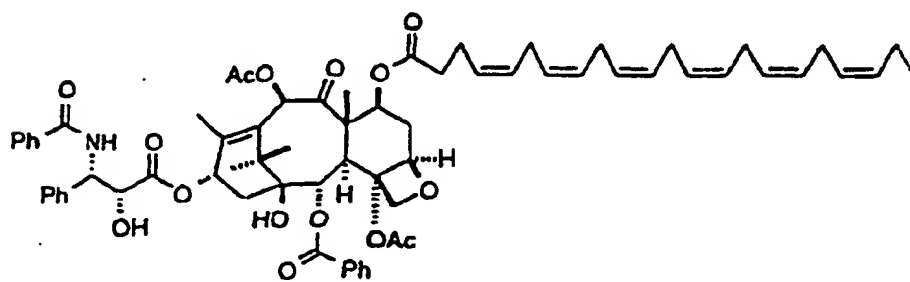
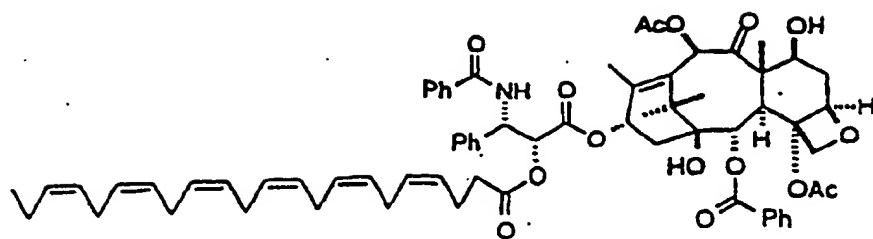
[0074] A long-term sustained release implant also may be used. "Long-term" release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well known to those of ordinary skill in the art and include some of the release systems described above. Such implants can be particularly useful in treating solid tumors by placing the implant near or directly within the tumor, thereby affecting localized, high-doses of the compounds of the invention.

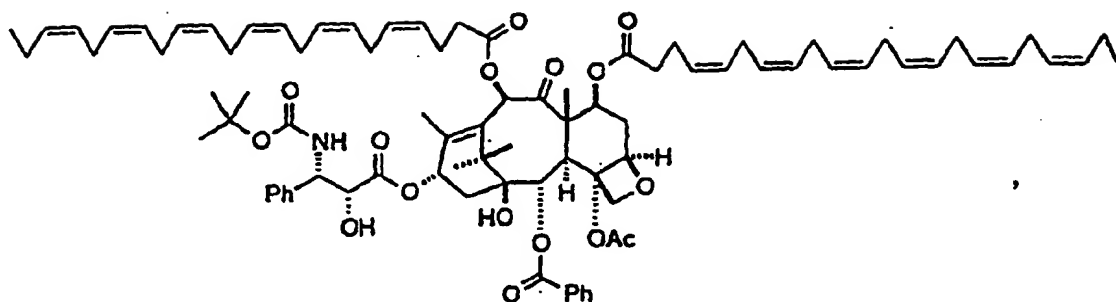
[0075] The conjugates of the invention also are useful, in general, for treating mammalian cell proliferative disorders other than cancer, including psoriasis, actinic keratosis, etc. They further are useful in treating diabetes and its complications, excess acid secretion, cardiovascular conditions involving cholesterol (e.g., hyperlipidemia and hypercholesterolemia), diarrhea, ovarian diseases (e.g. endometriosis, ovarian cysts, etc.) and as contraceptive agents.

[0076] Those skilled in the art will be able to recognize with no more than routine experimentation numerous equivalents to the specific products and processes described above. Such equivalents are intended to be included within the scope of the appended claims.

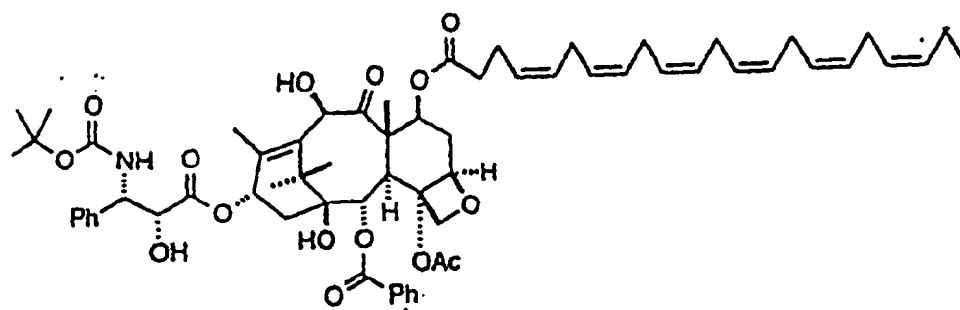
Claims

1. A composition for treating a condition in a noncentral nervous system tissue, comprising a covalent conjugate of *cis*-docosahexaenoic acid and a pharmaceutical agent effective for treating said condition, wherein the pharmaceutical agent is a taxane.
2. A composition as claimed in claim 1, wherein the *cis*-docosahexaenoic acid is conjugated directly to the pharmaceutical agent.
3. A composition as claimed in claim 1 or 2, wherein the tissue is breast, gastrointestinal or ovarian tissue.
4. A composition as claimed in any one of the preceding claims, wherein the taxane is Taxol or Taxotere.
5. A composition as claimed in claim 4, wherein the conjugate is

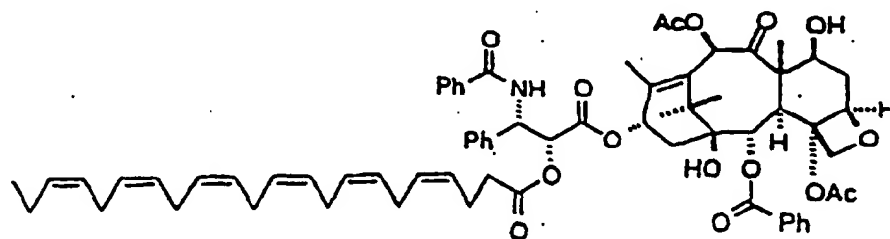


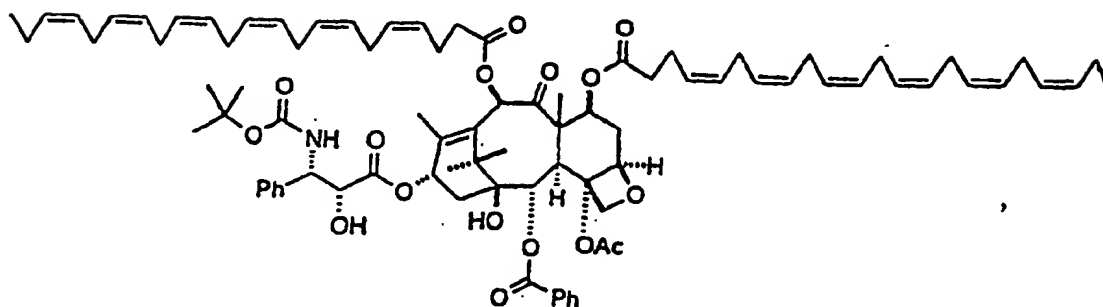
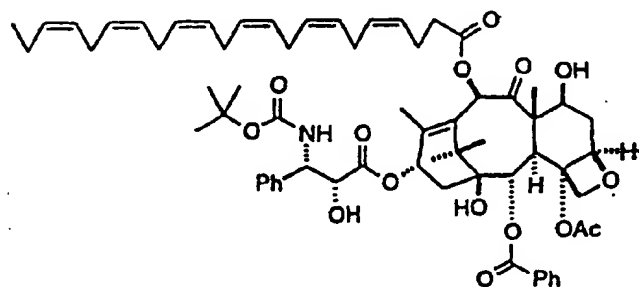
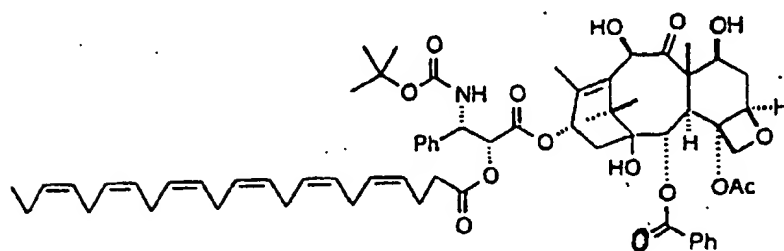
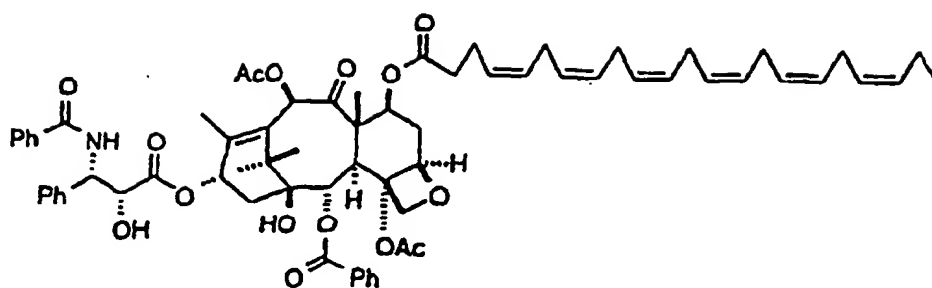


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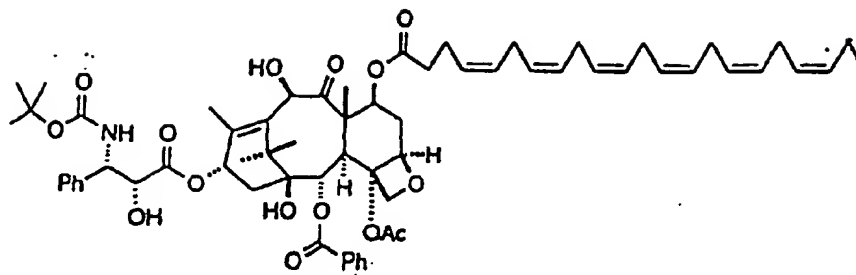


6. A composition as claimed in any one of the preceding claims wherein the condition is breast cancer, colon cancer or ovarian cancer.
7. A pharmaceutical agent comprising a covalent conjugate of *cis*-docosahexaenoic acid and a taxane, and a pharmaceutically acceptable carrier.
8. A pharmaceutical composition as claimed in claim 7, wherein the *cis*-docosahexaenoic acid conjugated directly to the taxane.
9. A pharmaceutical composition as claimed in claim 7 or 8, wherein the taxane is Taxol or Taxotere.
10. A pharmaceutical composition as claimed in claim 9, wherein the conjugate is



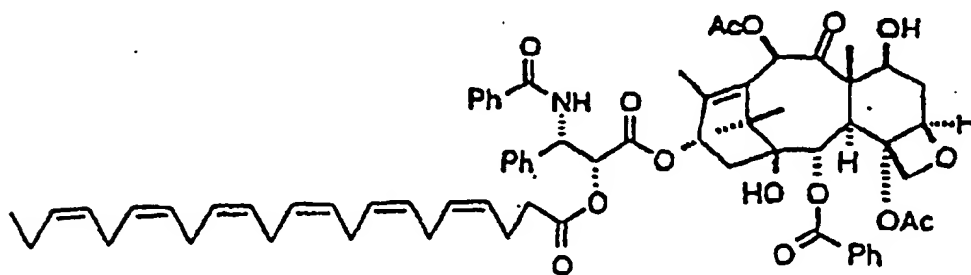


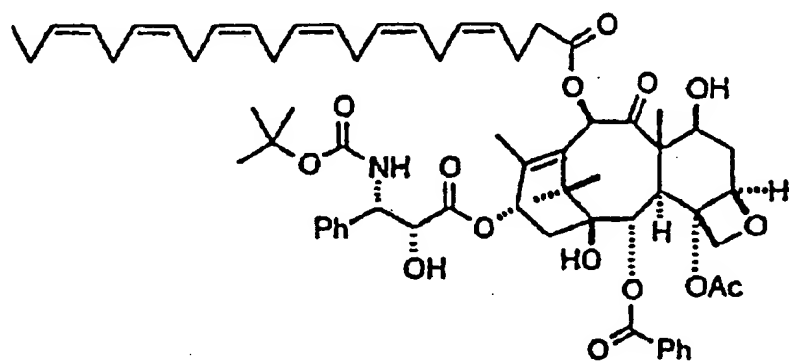
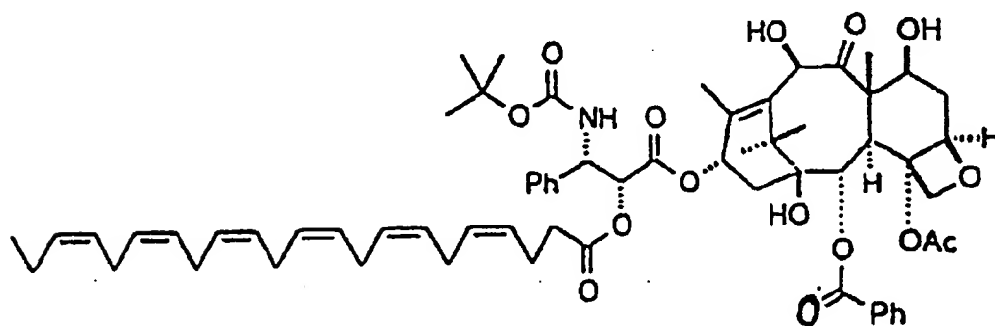
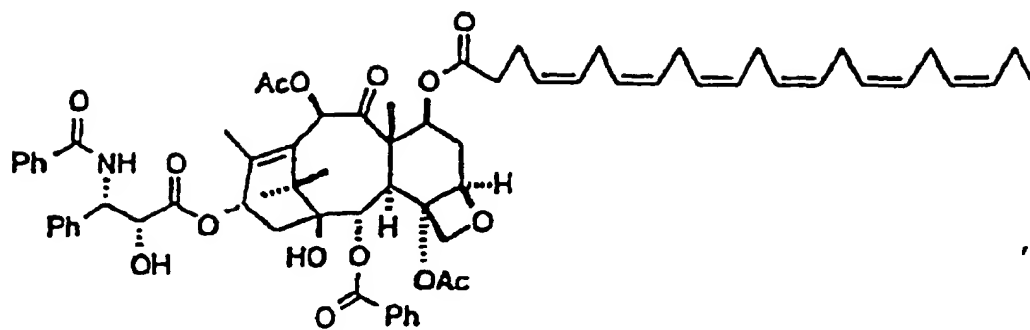
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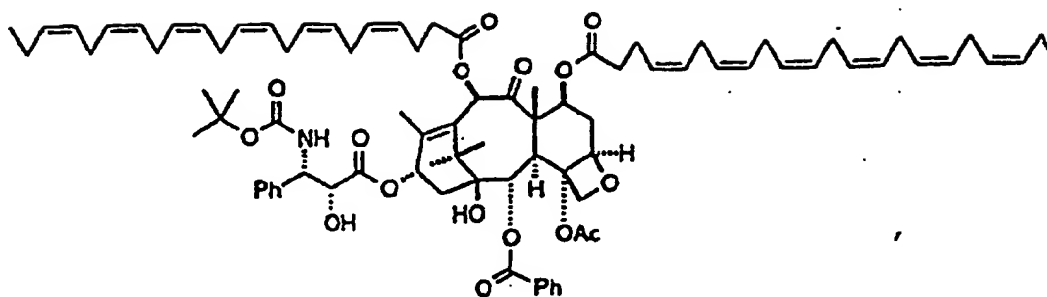


Patentansprüche

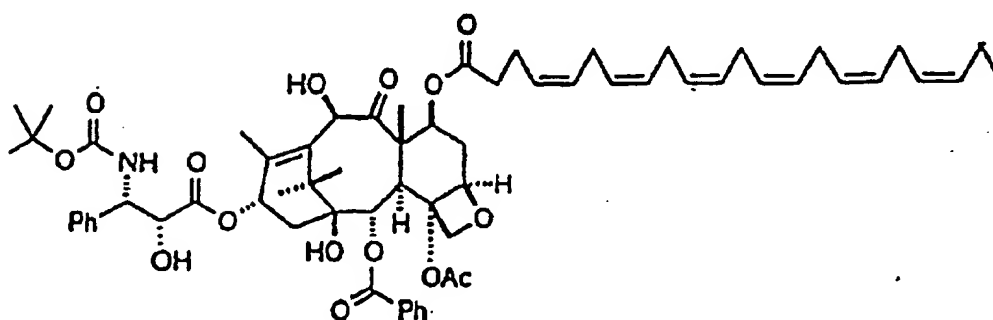
1. Zusammensetzung zur Behandlung eines Zustandes in einem Nicht-Zentralnervensystemgewebe, umfassend ein kovalentes Konjugat aus cis-Docosahexaensäure und einem zur Behandlung des Zustands wirksamen pharmazeutischen Mittel, wobei das pharmazeutische Mittel ein Taxan ist.
2. Zusammensetzung nach Anspruch 1, wobei die cis-Docosahexaensäure direkt mit dem pharmazeutischen Mittel konjugiert ist.
3. Zusammensetzung nach Anspruch 1 oder 2, wobei das Gewebe ein Brust-, Gastrointestinal- oder Ovarialgewebe ist.
4. Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei das Taxan Taxol oder Taxoter ist.
5. Zusammensetzung nach Anspruch 4, wobei das Konjugat





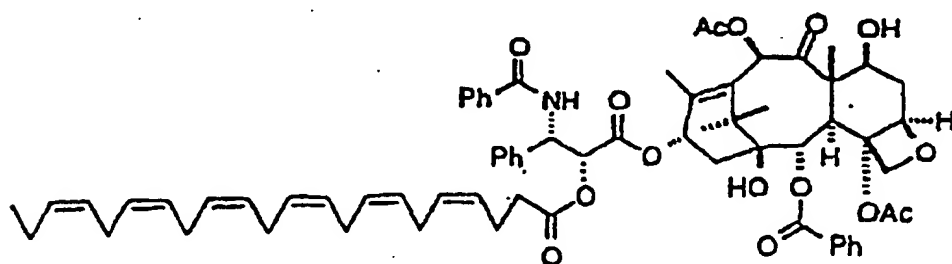


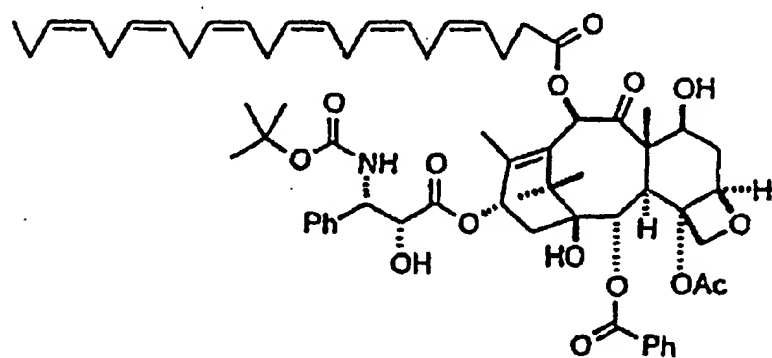
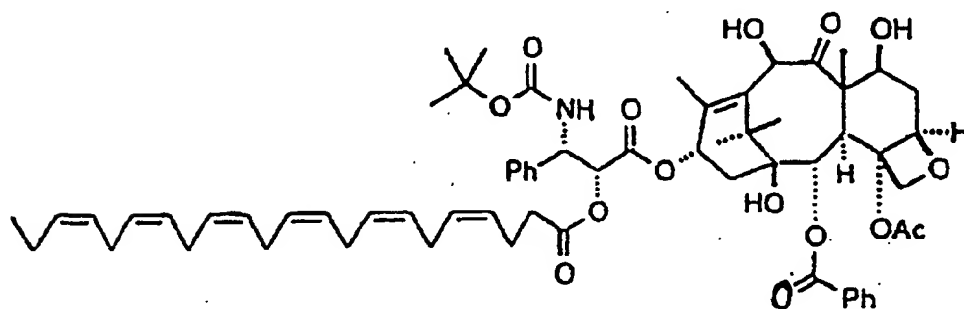
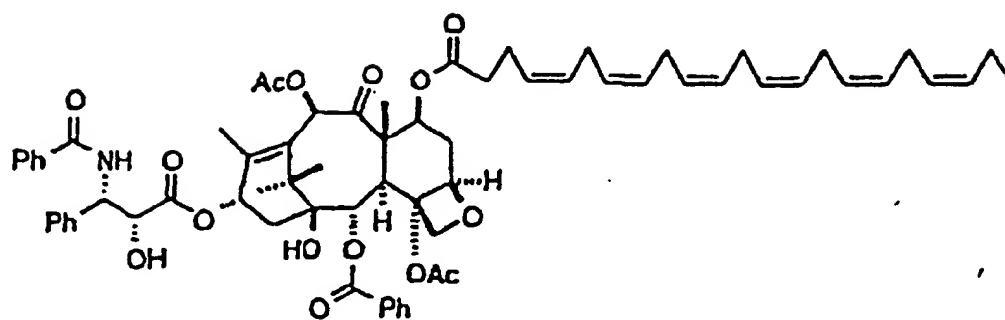
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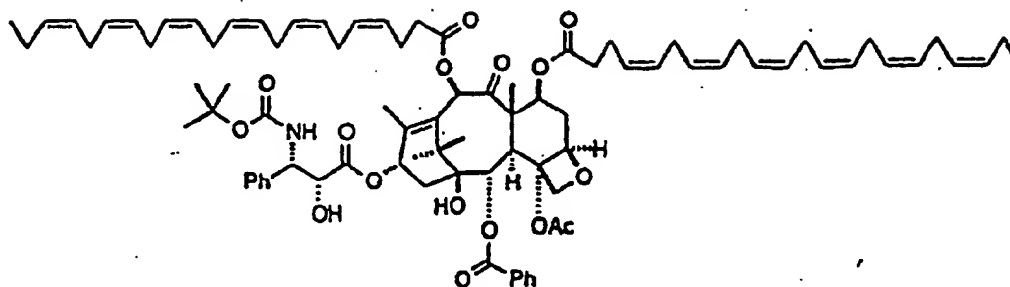


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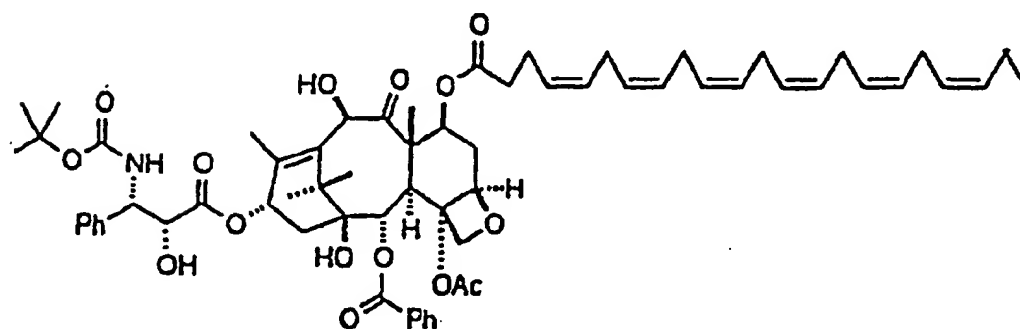
6. Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei der Zustand Brustkrebs, Kolonkrebs oder Ovarialkrebs ist.
7. Pharmazeutisches Mittel, umfassend ein kovalentes Konjugat aus cis-Docosahexaensäure und einem Taxan und einem pharmazeutisch annehmbaren Träger.
8. Pharmazeutische Zusammensetzung nach Anspruch 7, wobei die cis-Docosahexaensäure direkt mit dem Taxan konjugiert ist.
9. Pharmazeutische Zusammensetzung nach Anspruch 7 oder 8, wobei das Taxan Taxol oder Taxoter ist.
10. Pharmazeutische Zusammensetzung nach Anspruch 9, wobei das Konjugat







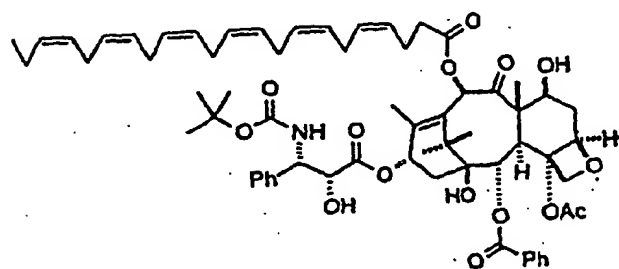
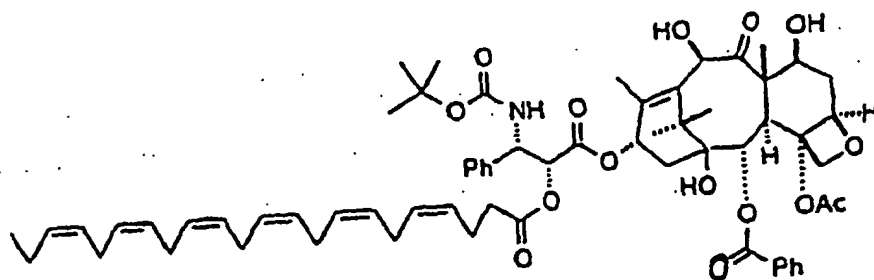
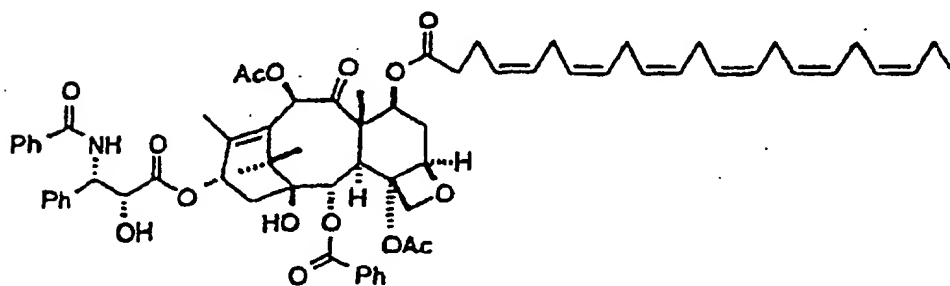
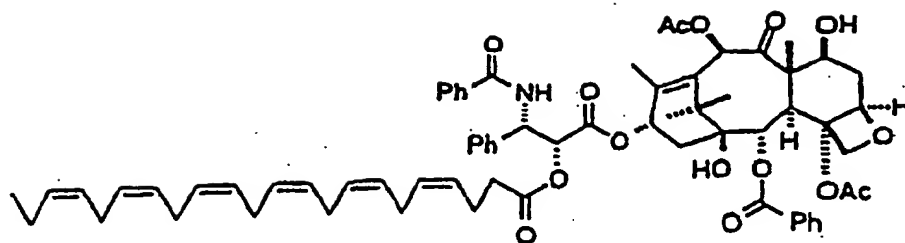
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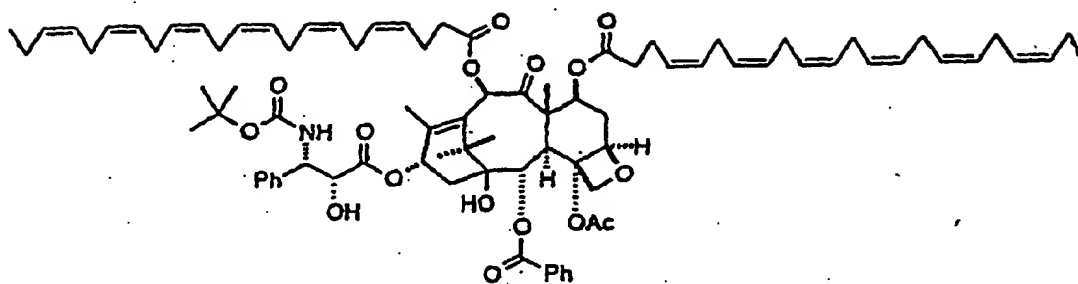


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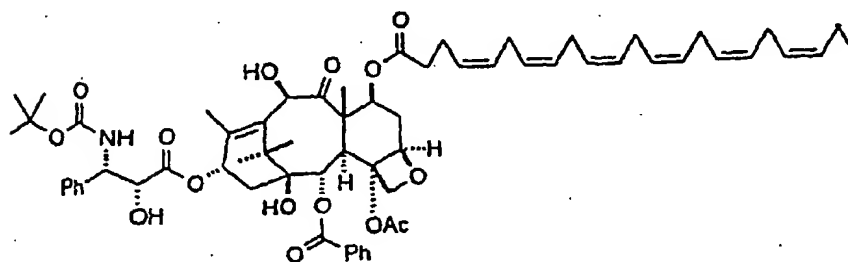
Revendications

1. Composition pour le traitement d'une affection dans un tissu n'appartenant pas au système nerveux central, comprenant un conjugué covalent d'acide cis-docosahexaénoïque et d'un agent pharmaceutique efficace pour le traitement de ladite affection, dans laquelle l'agent pharmaceutique est un taxane.
2. Composition suivant la revendication 1, dans laquelle l'acide cis-docosahexaénoïque est conjugué directement à l'agent pharmaceutique.
3. Composition suivant la revendication 1 ou 2, dans laquelle le tissu est le tissu mammaire, le tissu gastrointestinal ou le tissu ovarien.
4. Composition suivant l'une quelconque des revendications précédentes, dans laquelle le taxane est le taxol ou le taxotère.
5. Composition suivant la revendication 4, dans laquelle le conjugué consiste en

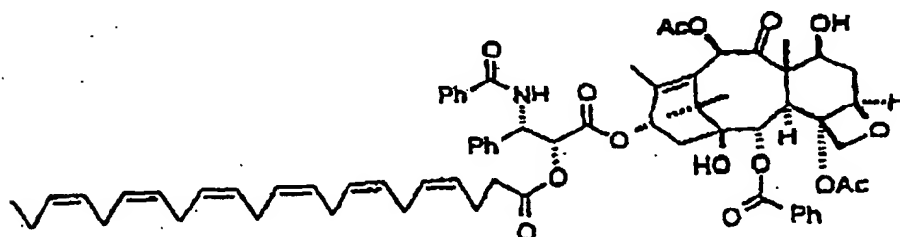


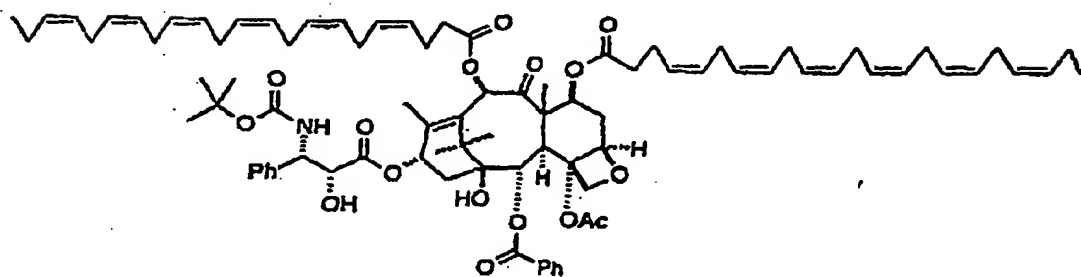
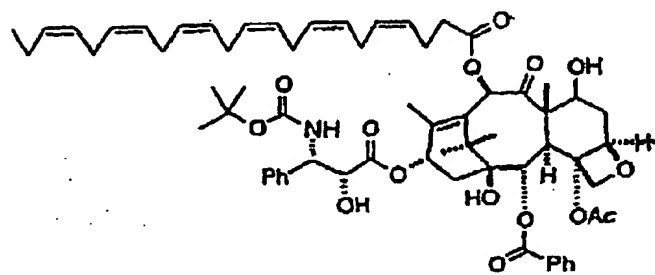
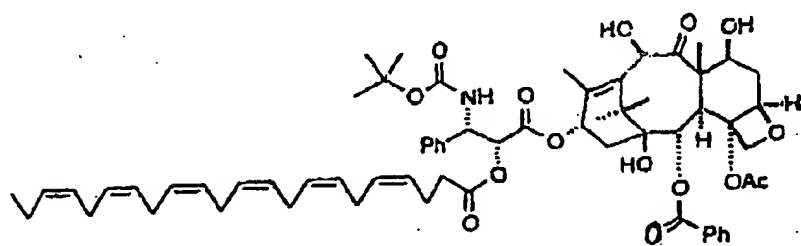
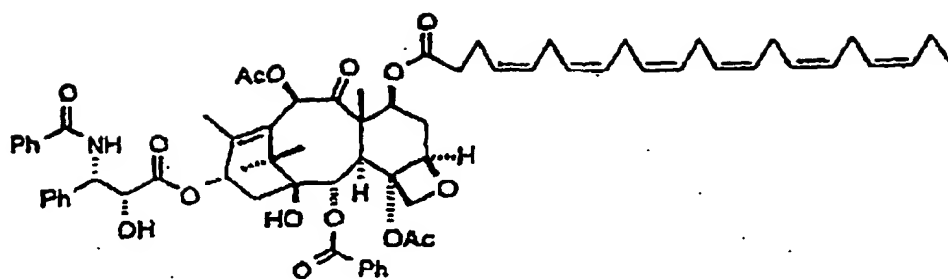


ou



6. Composition suivant l'une quelconque des revendications précédentes, dans laquelle l'affection est le cancer du sein, le cancer du côlon ou le cancer des ovaires.
7. Agent pharmaceutique comprenant un conjugué covalent d'acide cis-docosahexaénoïque et d'un taxane, et un support pharmaceutiquement acceptable.
8. Composition pharmaceutique suivant la revendication 7, dans laquelle l'acide cis-docosahexaénoïque est conjugué directement au taxane.
9. Composition pharmaceutique selon la revendication 7 ou 8, dans laquelle le taxane est le taxol ou le taxotère.
10. Composition pharmaceutique selon la revendication 9, dans laquelle le conjugué consiste en





OU

